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Sex Hormone Studies

The Effects on the Cellular Membrane Potentials and Contractility of Isolated Rat Atrium

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• The effects of estradiol, testosterone and progesterone on the electrical and mechanical characteristics of rat atria were determined. Cellular membrane potentials were obtained with microelectrodes and the contractility recorded from a sensitive strain gauge. All three steroids at concentrations near 10^{-5} M produced characteristic changes in the membrane potentials, the most striking effect being a pronounced slowing of the depolarization of the action potential, without simultaneously reducing the magnitudes of the resting or action potentials. As a result, there was slower impulse conduction in the atria, a lengthening of the action potential and a consequent increase in the refractory period. The repolarization rate was slowed. These changes are due to effects on the transmembrane fluxes of Na^+ and K^+, a decrease in permeability being assumed.

These effects are similar to those produced by the standard antiarrhythmic drugs, such as quinidine; and these steroids, particularly testosterone, have been found to be potent in the prevention and abolishment of atrial arrhythmias, both in vitro and in vivo. The steroids also block the effects of acetylcholine on the atria and this may play a role in the reduction in excitability and automaticity.

Testosterone, but not estradiol nor progesterone, exerts a temporary stimulation of the atrial contractility, which is not due to any effect on the membrane, but is related in some manner more directly to the contractile systems.

ALTHOUGH A GREAT DEAL is known about the effects of the male and female gonadal hormones, very few investigations of what part they play in cardiovascular actions have been made. Beyond the knowledge of their modification of the development of atherosclerosis and the indications that they have a vasodilating effect, very little is known of them in this regard. Particularly neglected is the question of possible direct influences in cardiac function. The reports on estradiol, progesterone and testosterone are not consistent, some indicating that they increase and some that they decrease myocardial contractility. Much of the work has been done in intact animals where direct mechanisms are difficult to distinguish from reflex and other compensatory phenomena. Electrocardiographic studies have demonstrated definite effects, but again it is difficult to decide whether these are direct or not. The present report is concerned with the direct effects of estradiol, progesterone and testosterone on the contractility and cellular membrane potentials of isolated rat atria, and with the bearing that these effects have on cardiac dysrhythmias.

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TABLE 1.—*Effects of Estradiol, Progesterone and Testosterone on Isolated Rat Atria*

Parameter	Estradiol (7.3×10^{-6} M) Mean of Controls	With Estradiol	Per Cent Change	Progesterone (6.3×10^{-6} M) Mean of Controls	With Progesterone	Per Cent Change	Testosterone (6.9×10^{-6} M) Mean of Controls	With Testosterone	Per Cent Change
Resting potential (mv.)	68.6	68.7	+ 0.1	72.1	72.1	0.0	67.6	67.5	− 0.1
Action potential magnitude (mv.)	79.9	79.6	− 0.4	83.8	82.4	− 1.7	77.8	78.7	+ 1.2
Overshoot (mv.)	11.3	10.9	− 0.4*	11.7	10.3	− 1.4*	10.2	11.2	+ 1.0*
Action potential duration (msec.)	42.4	46.8	+10.5	46.1	49.9	+ 8.2	49.7	61.3	+23.3
Action potential area (mv.-sec.)	1.13	1.29	+14.1	1.40	1.51	+ 7.9	1.36	1.79	+31.6
Depolarization rate (v./sec.)	60.4	47.7	−21.0	68.7	51.2	−25.5	63.6	44.4	−30.2
Repolarization rate (v./sec.)	1.27	1.16	− 8.7	1.19	1.07	−10.1	1.05	0.87	−17.1
Conduction rate (cm./sec.)	97.8	82.6	−15.5	89.9	68.7	−23.5	92.7	66.4	−28.4
Latent period (msec.)	18.7	21.9	+17.1	19.2	23.1	+20.3	18.7	22.3	+19.2
Developed tension magnitude (mg.)	458.0	410.0	−10.5	431.0	382.0	−11.3	429.0	436.0	+ 1.6
Developed tension duration (msec.)	64.2	61.4	− 4.4	68.4	66.2	− 3.2	71.4	67.2	− 5.8
Developed tension rise time (msec.)	40.6	39.0	− 3.9	41.3	39.5	− 4.3	41.4	39.0	− 5.8
Number of penetrations	180	100		242	120		255	125	
Number of atria	4	4		5	5		5	5	

*Change in overshoot given in mv.

mv. = millivolts; msec. = milliseconds; v = volts; sec. = seconds; mg. = milligrams; cm. = centimeters.

METHODS

The membrane potentials and mechanical activity of isolated rat atria electrically stimulated at a rate of 200 impulses per minute in Krebs-Ringer bicarbonate medium at pH 7.4 and 30° C. were determined, as reported previously[6] with microelectrodes and a sensitive strain gauge. The usual experiment consisted of the following four phases: (1) a 60-minute equilibration period, (2) a 30-minute control series of readings, (3) a 30-minute test series of readings following addition of the steroid, and (4) a 30-minute recovery series of readings following removal of the steroid from the bath. The effects were quite reversible so the test readings were compared with the means of the two control periods (steps 2 and 4, above) to calculate the per cent changes. Since ethanol was found to have effects on the atria, the steroids were dissolved in 50 per cent propylene glycol and added to the bath at a volume of 0.1 ml. A series of experiments showed that propylene glycol in that amount had no demonstrable action.

RESULTS

The responses of rat atria to testosterone, progesterone and estradiol at low concentrations are summarized in Table 1. Testosterone invariably produced a temporary positive inotropic effect during the first 10 to 15 minutes after its addition, but the contractile amplitude then returned to the normal value, or slightly below. This contractile stimulation was much more evident at higher concentrations; for example, 3.4×10^{-5} M produced an 80 per cent increase in contractile tension at 10 minutes.

The magnitudes of the resting and action potentials were altered insignificantly by the steroids, but the small increase in action potential was observed in each of the five experiments. The most striking changes in the electrical characteristics were slowings in the rate of depolarization and repolarization, leading to definite increases in the duration and area of the action potential. Each steroid produced the same type of effect, but testosterone was approximately twice as potent as estradiol or progesterone. The membrane electrical effects developed for 10 minutes following addition of the steroids and then remained relatively constant during the rest of the experimental period. After the steroid was washed out, it required 5 to 10 minutes for full recovery.

Acetylcholine is known to accelerate depolarization and repolarization rates, thus shortening the duration of the action potential, and this is probably a reflection of the altered rates of ionic fluxes through the atrial cell membranes. It might be expected that these steroids would antagonize these effects of acetylcholine and possibly reduce its action. This was found to be the case. Testosterone at 1.7×10^{-5} M antagonized the action of acetylcholine very effectively, a contractile depression of 68 per cent being reduced to 17 per cent. Indeed, after one to two minutes acetylcholine stimulated the atria in the presence of testosterone, so that an actual reversal of the acetylcholine effect was seen.

The significant slowing of the depolarization rate by testosterone, while simultaneously it increased the contractile tension, led us to investigate testosterone as an anti-dysrhythmic agent. Dysrhythmias such as atrial fibrillation are favored by two conditions: a shortening of the action potential (this reducing the refractory period) and an acceleration of the depolarization rate (this increasing the excitability). Testosterone on the isolated atria produced the opposite changes. Fibrillation was

induced in isolated rabbit atria (by high-frequency stimulation in a medium containing low K^+, low Ca^{++} and acetylcholine) and this was maintained for 20 minutes at rates between 800 and 1200 per minute. This fibrillation normally could continue indefinitely. After addition of 1.7×10^{-5} M testosterone, reversion to normal rhythm occurred in 10 to 15 minutes, and after 3.4×10^{-5} M testosterone in four to five minutes. During the period of conversion there was a gradual slowing of the rate. We are now continuing the testing of the anti-fibrillatory activity of testosterone in intact animals.

DISCUSSION

Some investigators have noted a positive inotropic, digitalis-like effect of the steroids on isolated and intact hearts[2,5] especially when the myocardium is hypodynamic. In the present work we noted a decided stimulation by testosterone in normally contracting atria. There is a definite difference between the steroids used; estradiol and progesterone produced only contractile depression. In addition, testosterone is more potent in slowing the rates of depolarization and repolarization. The contractile stimulation by testosterone does not appear to be related to the changes in membrane potentials, since the time courses of the changes were different, so that the positive inotropic action must be explained either by a more direct effect on the contractile mechanism or a facilitation of Ca^{++} influx.

A definite effect produced by all three steroids is the slowing of the rate of conduction. This follows from the slowed depolarization and reduced excitability of the myocardium. One would predict that, at high enough concentrations, these steroids might produce various degrees of cardiac block, but no sign of this was evident in the isolated atria.

The membrane potential changes produced by testosterone are similar to those reported for clinically useful anti-dysrhythmic drugs, such an quinidine.[7,11,12] It has also been found that steroids such as desoxycorticosterone and the spirolactones,[1,9] progesterone,[10] and estrogens[3,4] can be anti-dysrhythmic under certain circumstances. In the present studies, preliminary experiments on isolated rabbit atria showed a very pronounced anti-fibrillatory action of testosterone. Furthermore, conversion to a normal rhythm was obtained without any depression of the contractility; with quinidine a depression is always seen under comparable conditions. It is thus possible that testosterone or related steroids would be of some value clinically in the prevention or treatment of abnormal rhythms due to hyperexcitability of the myocardium. In addition, the effects of the various steroids on the heart may be able to explain some of the sex differences previously observed—for example, the susceptibility to cardiac dysrhythmias induced by epinephrine.[8]

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